degradation. MES in combination with HS can be a therapeutic stimuli for synovial joints by inducing HSP70 in articular cartilage.

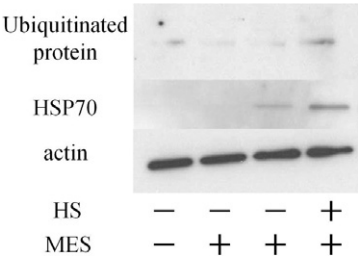

Fig. 1.

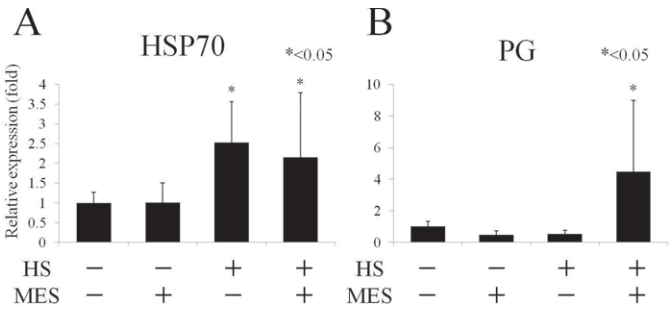

Fig. 2.

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EXPRESSION PROFILE OF CARBONIC ANHYDRASES IN ARTICULAR CARTILAGE

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Purpose: Carbonic anhydrases (CAs), which catalyze the reversible reaction of carbonate hydration, are important for cartilage homeostasis. The full spectrum of CA activity of all 13 isoenzymes in articular cartilage is unknown.
Methods: This study quantified an expression profile of CAs in rat articular cartilage, using quantitative polymerase chain reactions, and localized the CAs that were significantly expressed by chondrocytes in the zonal structure of cartilage using immunohistochemistry.
Results: Among the 13 functional CAs, CAs II, III, Vb, IX, XII and XIII were significantly expressed in articular cartilage. The expression of CA III spanned across the full thickness of articular cartilage. CA IX was limited in the superficial zone of cartilage and CA XIII expressed in the superficial and partially mid zone. CA II was seen in the mid and deep zone. CA XII was more restricted in the deep zone and CA Vb was found in the deep zone and subchondral bone.
Conclusion: Since CAs play a role in mineralization and demineralization, these results provide a framework for understanding individual CAs as well as the integrated CA family in cartilage biology and pathology.

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SUPERFICIAL CHONDROCYTES FROM OSTEOARTHRITIS PATIENTS HAVE REDUCED MATRIX METALLOPROTEINASE EXPRESSION FOLLOWING COMPRESSION

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Purpose: Articular cartilage functions as a shock absorber in diarthrodial joints. During joint loading, the superficial (S) zone of the cartilage can be subjected to compressive strains of up to 50%. When damaged, cartilage degeneration occurs, eventually leading to osteoarthritis (OA). Cartilage degradation usually starts from the superficial zone, reaching the deeper zones as the disease progresses. Matrix metalloproteinases (MMPs) are collagenases that are up-regulated following cartilage injury and play a role in matrix degradation and OA. We hypothesize that S chondrocytes from macroscopically normal parts of the OA joints can be used to model early-stage OA, and that loading will affect the production of MMPs. In this study, we investigated the effects of dynamic compression on S chondrocytes obtained from OA patients, and its influence in modulating MMP production.
Method: Human S chondrocytes from the macroscopically normal parts of 3 osteoarthritic joints (P2) were encapsulated in 2% alginate and pre-cultured in serum-free chondrogenic media supplemented with TGF-β1 for 2 weeks. Following pre-culture, constructs were dynamically compressed (1 Hz, 50% strain, 3hr) for 2 weeks. For the analysis of MMPs, conditioned media were collected at each media change. Concentrations of MMP-3 and MMP-13 were measured using a multiplex ELISA (Millipore). After 2 weeks of compression, S constructs were embedded in paraffin for immunofluorescence staining of collagen types II, IV, VI, and IX.
Results: Dynamic compression reduced MMP production by S chondrocytes compared to the controls with no loading. Without compressive stimulation, S chondrocytes on average produced higher levels of MMP-3 (~250-fold) and MMP-13 (~2.4-fold). Immunofluorescence images also showed brighter staining of collagen types II, IV, VI, and IX in S constructs subjected to compression compared to those with no loading.
Conclusions: Mechanical load is a potent modulator of chondrocyte expression of MMPs, and hence cartilage homeostasis. While injurious loading can initiate cartilage degradation and OA, absence of mechanical stimulation can also result in elevated levels of MMPs, cartilage thinning and proteoglycan loss in vivo. Our in vitro model was able to detect major decreases in MMP expression by S chondrocytes from OA patients with moderate in vitro loading. These data highlight the importance of appropriate mechanical stimulation in maintaining cartilage matrix, and also implicate loading as an important parameter to optimize when engineering articular cartilage from OA chondrocytes.

Cartilage Repair and Mesenchymal Cells

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ANTI-FIBROTIC EFFECT OF ADIPOSE STROMAL CELLS IN COCULTURE WITH CHONDROCYTES FROM OSTEOARTHRITIC PATIENTS

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Purposes: Osteoarthritis (OA) characterized by degeneration of articular cartilage is the most frequent rheumatic disease. Mesenchymal stem cells (MSC) isolated from bone marrow (MSC) or adipose tissues (adipose stromal cells (ASC)) secrete a large amount of factors with immunomodulatory, proliferative, anti-fibrotic or anti-apoptotic properties. The possibility that these cells, through their trophic potential, may influence the course of chronic degenerative disorders and prevent cartilage degradation is promising for the treatment of OA. The aim of our work was to evaluate the effects of ASC or MSC on OA chondrocyte phenotype in vitro.
Methods: OA ASC were isolated from intra-articular (Hoffa-ASC) or hip (hip ASC) subcutaneous adipose tissue and healthy ASC from abdominal depot (abdo-ASC). MSC were obtained from healthy and OA donors. ASC or MSC were co-incubated with OA chondrocytes cultured either in monolayer or in pellet during 2 or 7 days using cell culture inserts. We evaluated the specific markers of mature chondrocytes (collagen IIB (col IIB), aggregan (Agg), link and sox9), hypertrophic chondrocytes (MMP13, collagen X and alkaline phosphatase (AP)) and fibroblasts (collagen I and III) by RT-qPCR analysis. Secreted factors were quantified by ELISA.
Results: After 2 or 7 days, chondrocytes co-cultured in pellet with abdo-ASCs exhibited no change in the expression level of the markers tested. On the contrary, in monolayer, abdo-ASC induced a significant decrease of col IIB, MMP13 and col I expression in chondrocytes at D2. After 7 days, we observed a stable expression of the markers specific for mature chondrocyte and a diminution of MMP13, AP, Col I and Col III. Compared to abdo-ASCs, Hoffa-ASC and Hip-ASC behaved differently. They reduced both mature chondrocyte makers and hypertrophic/fibrosis markers. When comparing MSC from healthy subjects, we measured a decrease of Agg, Link and Sox9 expression and stable levels of hypertrophic/fibrotic markers in OA chondrocytes whereas MSC from OA patients maintain chondrocyte marker expression and reduce hypertrophic/fibrotic markers (MMP13, Col I and Col III). Finally, factors known to be involved in fibrosis and matrix remodeling (HGF, TIMP-1 and -2, MMP-1 and -9, IL1-RA, IL1β

and TNFα) were quantified in culture supernatants by ELISA. We observed that the anti-fibrotic factor HGF was not secreted by chondrocytes or ASC alone but its secretion was induced after 7 days of co-culture.
Conclusions: We have set up optimal co-culture conditions for evaluating the effect of ASC and MSC on the OA chondrocyte phenotype. The results suggest that the main effect of ASC from abdominal subcutaneous fat and MSC from OA donors is to maintain the chondrocyte phenotype by reducing their dedifferentiation as observed by the decrease of fibroblast-associated markers. This effect is at least partly due to the induction of HGF secretion which is a known anti-fibrotic factor. Interestingly, this anti-fibrotic effect seems to depend on the cell origin and pathological status.

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DOXYCYCLINE CONTROL OF LOCALIZED *IN VIVO* TRANSGENE EXPRESSION IN CARTILAGE INJURED JOINTS

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Purpose: Efforts to delay osteoarthritis through improving treatment of damaged articular cartilage remains challenging. Sustained intra-articular delivery of therapeutic agents that can promote cartilage repair is of high potential clinical importance. Intra-articular injection of adeno-associated virus (AAV) has been used in clinical trials for delivery of bioactive substances. Methods to localize and control *in vivo* transgene expression are important to enhancing the therapeutic efficiency and safety of intra-articular gene therapy. This study tests the hypotheses that (1) *in vivo* AAV transgene expression localizes to cartilage defects, and that (2) *in vivo* transgene signal can be externally controlled using oral doxycycline.
Methods: Longitudinal *in vivo* study was performed using twelve male Sprague-Dawley rats with surgically created unilateral full thickness cartilage injuries. To characterize the localization and persistence of transgene expression, six rats received a single intra-articular injection of AAV2-CMV-Luc one week after surgery to both injured and uninjured stifles. To characterize the controllability of the transgene expression, six rats received a single injection of AAV2-tetracycline response element (TRE)-Luc one week after surgery to both injured and uninjured stifles. Rats injected with the tetracycline controllable AAV2-TRE-Luc received doxycycline in the drinking water beginning 14 days after AAV injection for one week. Doxycycline was then removed from the drinking water. Luciferase expression was evaluated over a 6-month period in both groups of rats using the IVIS® Live Animal Imaging System (Xenogen). At the time of sacrifice, stifle joints were opened and luciferase expression was evaluated to localize intra-articular transgene expression.
Results: Longitudinal evaluation of in vivo luciferase expression in rats receiving AAV2-CMV-Luc using live animal imaging revealed transgene expression from 7 days post-injection. Luciferase transgene expression persisted and remained stable throughout the 6 months study. When the joints were opened, luciferase expression was found to be highly localized to the vicinity of the cartilage defect and associated repair tissue. Rats that received the inducible AAV2-TRE-Luc in the injured joint showed gene expression upregulation with addition of doxycycline ($p < 0.05$) and downregulation of luciferase following removal of doxycycline ($p < 0.05$) to the drinking water.
Conclusions: Longitudinal *in vivo* results showed persistent and stable AAV2-mediated gene expression, which was localized to the vicinity of the cartilage injury. Furthermore, this highly localized *in vivo* intra-articular transgene expression was controllable by oral administration of doxycycline. These data support further study of the in vivo therapeutic potential of AAV for safe and localized delivery of bioactive substances to improve cartilage repair following injury as a strategy to prevent or delay the onset of osteoarthritis.

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ARTICULAR CARTILAGE REGENERATION AND EAR-WOUND HEALING IN GENETIC MOUSE MODELS

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Purpose: Articular cartilage has limited self-regeneration ability and, once damaged leads to further cartilage degeneration and predisposes to the development of osteoarthritis. It has been found that certain mouse strains (MRL/MpJ, DBA/1) can regenerate their full-thickness articular cartilage defects. These and another strain, LG/J, can also heal through-and-through ear puncture wounds unlike C57BL/6 and SM/J strains. MRL/MpJ and LG/J share 75% of their genome. It might indicate that the healing ability in certain strains might be linked to their specific genetic composition. To test this hypothesis, we developed a genetic mouse model of articular cartilage regeneration and ear-wound healing using recombinant inbred lines. These lines were generated from LG/J (healer) and SM/J (non-healer) strains with each line containing unique portions of the healing strain genome. We aim (i) to identify cartilage regeneration and ear-wound healing phenotypes and (ii) to establish the relationship between tissue regeneration (ear-wound healing) and cartilage regeneration in these strains.
Methods: A total of nine recombinant inbred lines, parental and control strains were used in this study. An acute full-thickness cartilage injury was introduced through microsurgery in the trochlear groove of both knees of 8-weeks old mice ($n = 350$). Knee joints were harvested at indicated time points. Paraffin embedded blocks were sagittally sectioned and stained with toluidine blue. Slides were graded for five parameters on a scale from 0 to 14 for cartilage regeneration. Selected sections from each strain were stained for collagen types I and II. For the ear-wound phenotype, a 2-mm through-and-through puncture was made in mice ($n = 418$) at 6-weeks of age and healing outcomes measured after 4 weeks.
Results: Results showed that MRL/MpJ healed at 12 weeks while LG/J and strain 6 showed healing trend only at 16 weeks (Fig. 1).

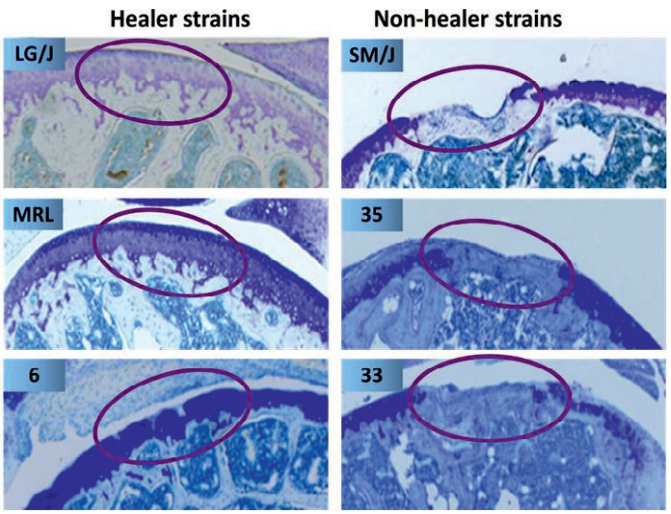

Fig. 1. Representative sagittal sections of cartilage repair phenotypes in healer and non-healer strains.

All other recombinant inbred lines failed to regenerate cartilage at 12 or 16 weeks post-surgery. Similarly SM/J, C57BL/6 and surprisingly DBA/1 and DBA/2 strains did not heal (Fig. 2).
The neo-cartilage in healer strains (MRL/MpJ, LG/J and 6) showed type II collagen (hyaline-like) and proteoglycan deposition. For ear-wound healing, in addition to strains 6, MRL/MpJ and LG/J, strains 19, 35 and 46 also showed healing of ear wounds at 4 weeks (Fig. 3).
Conclusions: We conclude that cartilage regeneration is a strain dependent phenomenon and is not always associated with ear-wound healing. It is also tempting to speculate that a distinctive set of genes might be involved in these processes. Overall, our study will allow us to